S4.17 The activity of exogenous NAD(P)H dehydrogenases in *Acanthamoeba castellanii* mitochondria

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The mitochondrial respiratory chain of plants and fungi contains multiple NAD(P)H dehydrogenases. In addition to Complex I there are two rotenone-insensitive dehydrogenases located on the outer surface of the inner mitochondrial membrane (ND_{ex}), i.e., external NADH and external NADPH dehydrogenases. Using bioenergetic methods (oxygen consumption and membrane potential measurements) we have investigated protozoan *A. castellanii* mitochondria in order to study the activity of ND_{ex}. We have determined the activity of both NADH and NADPH dehydrogenases with the maximum value at pH 6.8, likewise the cyanide-resistant alternative oxidase activity. It seems to be consistent with the putative role of these enzymes which probably cooperate with each other and very likely constitute a wasteful system preventing overreduction of the electron transport components in the respiratory chain. We have also examined a Ca^{+2}-dependence of *A. castellanii* ND_{ex} activities. Our data show that NADH dehydrogenase is probably slightly or not sensitive to Ca^{+2} ions in contrast to NADPH dehydrogenase, which is Ca^{+2}-sensitive and this sensitivity increases with the rise of pH value.

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S4.18 NADH induced conformational changes in *E. coli* complex I measured by site-directed spin-labelling

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We have probed the extent of local structural changes of the *E. coli* complex I upon the addition of nucleotides by site-directed spin-labelling. Surface cysteine variants of complex I have been generated, prepared and spin-labelled with a sulfhydryl-specific nitroxide reagent. The NADH:decyl-ubiquinone activity of the labelled enzyme variants was unchanged compared to the wild type enzyme, indicating the negligible influence of the label on the physiological activity. From the line shape of the spectra, the spin-label mobility was deduced, which is a direct measure of the local protein environment. All measured mobilities of the nitroxide side chains reflected the fold of the protein as deduced from the crystal structure and secondary structure prediction. For sites where a restricted mobility of the label indicated contacts with neighbouring amino acids, a change in the labels EPR-spectrum was observed upon the addition of NADH, whereas the addition of NAD^+ had no effect. Therefore we were able to pinpoint the observed conformational changes of the *E. coli* complex I to distinct secondary structure elements within individual subunits.

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S4.19 Assembly of the *Escherichia coli* NADH:ubiquinone oxidoreductase (complex I)

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The bacterial complex I generally consists of 14 different subunits. In contrast to the mitochondrial complex, where several distinct assembly intermediates were detected, the assembly of the bacterial complex was not examined until now. We used *Escherichia coli* strains in which the *nuo*-genes coding for the subunits of complex I are individually disrupted by insertion of a resistance cartridge and studied the assembly of the complex in the mutants. The cytoplasmic membranes of these mutants exhibited no complex I specific activity. However, in the cytoplasm of some mutants the subunits and cofactors of the soluble NADH dehydrogenase fragment were detected. The mutants lacking either *nuoK* or *nuoL* contain a larger membrane-bound complex I fragment, which exhibits NADH/ferricyanid but no physiological activity. The fragment from the *nuoL* mutant strain was purified and characterised. The role of NuoL in the mechanism of complex I is elucidated by the activities of this partially assembled complex I.

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S4.20 Substrate crosstalk involving conformational changes in *E. coli* respiratory complex I

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The respiratory complex I couples the transfer of electrons from NADH to ubiquinone with the translocation of protons across the membrane. An addition of NADH but not NADPH to the *E. coli* complex I led to conformational changes identified by electron microscopy and CD-spectroscopy. EPR spectroscopy revealed that both, NADH and NADPH, completely reduce the Fe/S-clusters of the enzyme. However, the enzymatic activity with NADPH is, in contrast to the activity of NADH, not coupled with proton translocation and not sensitive to inhibitors of the quinone site. Instead, the reaction with NADPH led to an increased production of superoxide radicals. We propose, that the binding of NADH and not the reduction of the enzyme leads to conformational changes needed for opening the ubiquinone binding site.

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S4.21 Monitoring the conformational change of the NADH dehydrogenase fragment from the *E. coli* complex I by attenuated total reflexion spectroscopy

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